Genetic risk factor characterizes abdominal aortic aneurysm from arterial occlusive disease in human beings: CCR5 Δ32 deletion

Giorgio Ghilardi, MD,[a] Maria Luisa Biondi, MD,[b] Lodovica Battaglioli, MD,[b] Antonella Zambon, PhD,[c] Emma Guagnellini, MD,[b] and **Roberto Scorza, MD,[a]** *Milan, Italy*

Objective: **Inflammation is involved in the pathogenesis of atherosclerosis and abdominal aortic aneurysm (AAA), and chemokines are mediators of the inflammatory process. The homozygous Δ32 deletion mutation of the gene of the chemokine receptor CCR5 is a cause of its lack in inflammatory cells. The purpose of this study was to investigate the relationship between CCR5 Δ32 deletion mutation and AAA, peripheral arterial occlusive disease (PAOD), and carotid stenosis.**

Methods: **The CCR5 Δ32 polymorphism was genotyped in 380 subjects: 70 patients operated on to treat AAA (21 ruptured AAAs, 49 elective repair), 76 patients with PAOD, 62 patients with carotid stenosis, and 172 age-matched and sex-matched healthy control subjects. Risk factors for AAA were considered. Each patient was assessed according to a diagnostic procedure tailored to symptoms at presentation.**

Results: **In patients with AAA the Δ allelic variation was significantly different compared with control subjects ($P = .002$; odds ratio [OR], 2.7; 95% confidence interval [CI], 1.41-5.15). The increased presence of this allele differentiates AAA from both PAOD ($P = .017$; OR, 2.77; 95% CI, 1.17-6.52) and carotid stenosis ($P = .01$; OR, 3.47; 95% CI, 1.31-9.11). The presence in the genotype of patients with AAA of at least 1 Δ32 allele is more frequent in ruptured AAAs than in electively repaired AAAs (genotype: OR, 4.04; 95% CI, 1.34-12.1; $P = .011$; allelic frequency: OR, 2.75; 95% CI, 1.07-7.07; $P = .035$). Among the patients, multiple regression analysis showed that the Δ32 allele is an independent risk factor for AAA vs PAOD (OR, 3.13; 95% CI, 1.33-7.33; $P = .012$) and for ruptured AAAs vs electively repaired AAAs (OR, 3.52; 95% CI, 1.01-11.80; $P = .045$).**

Conclusions: **CCR5 Δ32 deletion mutation is significantly more frequent in patients with AAA than in control subjects and in both patients with PAOD and carotid stenosis, and could be a factor that differentiates AAA from PAOD, and ruptured AAAs from AAAs that can be electively repaired. (J Vasc Surg 2004;40:995-1000.)**

Clinical Relevance: **The major threat of abdominal aortic aneurysm (AAA) is rupture, accounting for extremely high mortality. This occurrence has been correlated to aneurysm size, but it is a common observation that small AAAs can rupture and large AAAs can remain stable for many years. This study was carried out in an attempt to search for genetic markers of aneurysm rupture. Some single nucleotide polymorphisms are implicated in acceleration of transcription for enzymes involved in the inflammatory process and in extracellular matrix remodeling. An association between single nucleotide polymorphisms and aneurysm rupture could enable better selection for surgical indications in patients with small AAAs and in patients at poor risk with large AAAs.**

Abdominal aortic aneurysms (AAA) are common in the aging populations of industrialized nations, with estimates of prevalence as high as 10%. AAA rupture accounts for approximately 4% of all deaths in persons older than 65 years.

Despite the high risk for death associated with AAA, few definitive risk factors have been identified. The sequence of biochemical and cellular events in the evolution of AAAs in human beings is largely unknown. AAA is often associated with atherosclerosis; however, there are certain pathogenetic, epidemiologic, and genetic differences between the 2 diseases.[1,2] The basic phenomena in the pathogenesis of AAA are degradation of the extracellular matrix components (elastin, collagens) and loss of the structural integrity of the aortic wall.[1,2] AAA disease typically involves tissue inflammation, as noted by the presence of inflammatory leukocytes and various cytokines, which are considered relevant in the immunopathogenesis of AAA, leading to destruction of the aortic matrix.[1-3] Prominent inflammatory infiltrates of macrophages and lymphocytes are found in both peripheral arterial occlusive disease (PAOD) and AAA.[4,5]

Chemokines are cytokines that promote migration of leukocytes into inflammatory sites, and their differentiation or activation.[6] In recent years their role in the pathogenesis of several immune-mediated diseases has been recognized.[7] Moreover, polymorphisms in gene encoding chemokines and their receptors (ie, CCR5, CCR2, stromal cell–derived

From Dipartimento MCO,[a] Clinica Chirurgica Generale, Università degli Studi di Milano–Polo Didattico S. Paolo, Laboratorio di Chimica Clinica e Microbiologia Ospedale S. Paolo–Polo Universitario,[b] and Dipartimento di Statistica,[c] Facoltà di Scienze Statistiche, Università degli Studi di Milano-Bicocca.

Competition of interest: none.

Reprint requests: Giorgio Ghilardi, MD, Dipartimento MCO Clinica Chirurgica Generale, Università degli Studi di Milano–Polo S. Paolo, Via A. Di Rudinì, 8 I-20142 Milano, Italia (e-mail: giorgio.ghilardi@unimi.it).

0741-5214/$30.00

doi:10.1016/j.jvs.2004.08.014

factor) influence the course of human immune deficiency virus infection and inflammatory diseases.[8-10]

CCR5 is a chemokine receptor that could potentially be involved in plaque development. CCR5 is expressed on macrophages, T cells, aortic smooth muscle cells, and coronary endothelial cells[11-14]; CCR5 ligands have been detected in plaques.[15] Furthermore, recent genetic screening studies showed that natural deficiency in CCR5 protects against early myocardial infarction[16] and severe coronary artery disease.[17] A common 32-base pair deletion mutation in the CCR5 gene (Δ32) that causes truncation and loss of CCR5 receptors on lymphoid cell surfaces of homozygotes was recently described.[8]

The purpose of this study was to compare CCR5 genotypes between patients with AAA, and patients with PAOD or internal carotid artery stenosis.

MATERIAL AND METHODS

The present study included 4 groups of patients consecutively recruited during routine examination through the year 2002: 70 patients with AAA, 76 patients with PAOD, and 62 patients with carotid stenosis.

Control subjects consisted of 172 volunteers consecutively recruited in the same period from outpatients referred to our vascular laboratory who did not have clinically or ultrasound-detected atherosclerosis.

Within the group of patients with AAA, 21 were consecutively admitted to the Emergency Department of San Paolo Hospital, Milan, Italy, with ruptured aneurysms (ruptured AAA group). Forty-nine patients with AAA (elective AAA group) and all patients with PAOD or carotid stenosis were consecutively admitted to our vascular surgery unit. Each patient, with the exception of the ruptured AAA group, was assessed according to a diagnostic procedure tailored to symptoms at presentation. Once assessment of the admission disease was completed, noninvasive ultrasound evaluation of other vascular regions was performed.

Doppler ultrasound scanning (color flow Doppler imaging) was used to assess the abdominal aorta and the carotid, renal, iliac, femoral, popliteal, and tibial arteries. When appropriate, coronary flow was assessed with stress electrocardiography, echocardiography, and myocardial scintigraphy. All patients with hypertension underwent routine renal artery echo Doppler ultrasound scanning.

Inclusion criteria were as follows: (1) Asymptomatic vascular areas were included when arterial stenosis exceeding 50% was documented with color flow imaging. (2) Angiographic assessment of PAOD was carried out in patients with disabling or severe claudication (Fontain stages IIa and IIb), rest pain, or gangrene (Fontain stages III and IV). (3) To assess carotid stenosis, angiography or multiple-slice helical contrast material–enhanced computed tomography (angio-CT) was performed in patients who met the surgical criteria for color flow imaging. These criteria were set according to the American Heart Association guidelines for carotid endarterectomy.[18] (4) AAAs were assessed with both echo-tomography and contrast-enhanced CT. An aneurysm was defined as an increase of at least 150% in mean vessel diameter compared with the diameter of the immediately adjacent segment. Aneurysm diameter was measured on CT scans and compared with data on each surgical report.

Among the 76 patients with PAOD, Fontain disease stage was IIa in 21 patients, IIb in 38 patients, III in 15 patients, and IV in 4 patients.

According to the epidemiologic findings for AAA reported by Blanchard et al,[19] only cigarette smoking and arterial hypertension were considered risk factors for this disease.

Hypertension was defined according to the American Heart Association guidelines for the management of hypertension.[20] Smoker definition included both ex-smokers and active smokers.

Whole blood (3 mL) from patients and control subjects was collected into potassium ethylenediaminetetraacetic acid. DNA was prepared with an Istagene Matrix extraction kit (Bio-Rad Laboratories). The polymerase chain reaction for CCR5 was carried out in a total volume of 25 μL, with 5 μL of extracted genomic DNA; 100 μmol/L of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxythymodine triphosphate, and deoxycytidine triphosphate; 1.5 mmol/L of magnesium chloride; 1 unit of Taq polymerase; and the 2 primers, forward and reverse, each at a concentration of 80 nmol/L. The primers were designed with Primer Express software. The CCR5 primer sequence is forward primer 5′-CTGTGTTTGCGTCTCTCCCA-3′; reverse primer 5′-CCTCTTCTTCTCATTTCGACACCG-3′. The polymerase chain reaction was started with 10 minutes of incubation at 94°C to activate the enzyme, followed by 35 cycles of 20 seconds at 94°C, 20 seconds at 55°C, and 30 seconds at 72°C. The amplification was verified on agarose gel (3%).

Differences between groups were examined with the χ^2 test. Odds ratios (OR; approximate relative risk) were calculated as an index of the association of the CCR5 genotype with each phenotype. For each OR, 2-tailed probability values and 95% confidence intervals (CI) were calculated. Multivariate logistic regression was used to evaluate the strength of the association between Δ32 deletion and AAA, ruptured AAA in particular, to eliminate any possible confounding effects of hypertension, gender, or smoking. All statistical analyses were 2-sided, and were performed with Stata Statistical Software.

RESULTS

Demographic data and distribution of the 380 patients are shown in Table I. Gender frequency was different among the AAA, PAOD, carotid stenosis, and control groups. Hypertension was significantly more common in patients with AAA than in any other group (control subjects, $P = .0001$; carotid stenosis group, $P = .0047$; PAOD group, $P = .047$). Comparison of PAOD and carotid stenosis groups with the control group revealed that smoking and hypertension were significantly more common in patient groups than in the control group. The polymorphism distribution in patients and control subjects was in

Table I. Demographic data for patients with AAA, PAOD, or CS and for control subjects*

		PAOD		*CS*		*Control subjects*	
		t test	P	*t test*	P	*t test*	P
Age (y)							
AAA	71±6	1.714768	.088539	1.153986	.250627	2.398307	.017236
PAOD	68±9			0.488193	.6262	0	1
CS	69±8					0.645267	.519397
Control subjects	68±7						
		χ^2 *test*	P	χ^2 *test*	P	χ^2 *test*	P
Sex (M:F)							
AAA	61:9	2.8338	.0923	17.0081	<.0001	11.8424	.0006
PAOD	58:18	—		7.0874	.0078	3.0665	.0799
CS	34:28	—		—		2.0515	.1521
Control subjects	112:60	—		—		—	
Hypertension							
AAA	55/15	7.9863	.0047	3.9372	.0472	31.8535	<.0001
PAOD	43/33			0.5664	.4517	6.9809	.0082
CS	39/23					10.9475	.009
Control subjects	65/104						
Smoker (exsmoker)							
AAA	47 (7)/23	0.4726	.4918	0.4603	.4975	2.3849	.1225
PAOD	55 (3)/21			0.0008	.9779	5.6683	.0173
CS	45 (9)/17					5.0038	.0253
Control subjects	97 (3)/75						

AAA, Abdominal aortic aneurysm; *PAOD,* peripheral arterial occlusive disease; *CS,* carotid stenosis.
*Age, gender, hypertension, and smoking habits were considered risk factors and were compared among the 4 groups.

Table II. CCR5 genotype and frequency of Δ32 mutation in patients and control subjects

Genotype	*AAA*	*PAOD*	P	*Carotid stenosis*	P	*Control subjects*	P
WT/WT	51	67		56		152	
OR		1		1		1	
WT/Δ32	18	9		6		20	
OR		—		—		—	
Δ32/Δ32	1	0		0		0	
OR		—		—		—	
WT/Δ32 + Δ32/Δ32	19	9	.019	6	.01		.009
OR		2.77		3.57		20	
95% CI		1.07-7.52		1.20-11.39		1.31-6.06	
Δ32 frequency	.14	.06	.017	.05			.00
OR		2.64		3.27		2.7	
95% CI		1.09-6.84		1.20-10.28		1.32-5.47	

AAA, Abdominal aortic aneurysm; *PAOD,* peripheral arterial occlusive disease; *WT,* wild type; *OR,* odds ratio; *CI,* confidence interval.

accordance with the values predicted with the Hardy-Weinberg equilibrium model (Table II).

In patients with AAA the Δ32 allelic variation was significantly different compared with that in control subjects ($P = .002$; OR, 2.7; 95% CI, 1.32-5.47; AAA vs control subjects, Δ32 vs wild type [WT]). ORs associated with the presence of a genotype with at least 1 Δ32 allele (WT/Δ32 + Δ32/Δ32 vs WT/WT genotype) was 2.83 (95% CI, 1.32-5.47; $P = .0029$). Therefore the presence of the Δ32 allele seems to be more common in patients with AAAs than in control subjects.

The increased presence of this allele significantly differentiates AAA from both PAOD and carotid stenosis (Table II; OR, 2.77 and 3.47, respectively). No differences were observed in either allele or genotype distribution among patients with PAOD or carotid stenosis and control subjects.

Among the AAA group, patients with at least 1 Δ32 allele are at 4-fold higher risk for AAA rupture compared with WT/WT carriers (Table III).

No significant differences in aneurysm diameter were observed in WT and Δ32 allele carriers (WT/WT 53+/−22.3 mm vs WT/Δ32 + Δ32/Δ32 47.1+/−15.6 mm, mean ± SD; $P = .08$).

Multiple logistic regression analysis was performed to identify possible independent risk factors for AAA between patients and control subjects and between the AAA group and both the PAOD and carotid stenosis groups. The PAOD and carotid stenosis groups were considered as a whole, given the common characteristic of stenosis of the

Table III. CCR5 genotype and Δ32 mutation frequency in patients with ruptured and elective AAA repair

	Ruptured (N = 21)		*Elective repair (N = 49)*				
Genotype	*n*	*%*	*n*	*%*	*OR*	*95% CI*	P
WT/WT	11	52	40	82			
WT/Δ32	10	48	8	16			
Δ32/Δ32	0		1	2			
WT/Δ32 + Δ32/Δ32	10	48	9	18	4.04	1.13-14.30	.011
Δ32 frequency	0.24		0.10		2.75	0.92-8.08	.035

WT, Wild type; *OR,* odds ratio; *CI,* confidence interval.

Table IV. Multiple logistic regression analysis

	Variable	*OR*	*95% CI*	P
AAA vs control subjects	Δ32	2.17	0.95-4.96	.064
	Cigarette smoking	2.56	1.01-9.27	.047
	Hypertension	5.06	2.94-10.94	.001
	Sex (male)	4.02	1.56-3.78	.002
AAA vs PAOD + CS	Δ32	3.13	1.33-7.33	.012
	Hypertension	2.54	1.15-5.60	.008
	Cigarette smoking	1.65	0.74-3.65	.21
	Sex (male)	3.26	1.36-7.81	.008
Ruptured vs elective AAA repair	Δ32	3.52	1.01-11.80	.045
	Cigarette smoking	0.48	0.11-2.05	.325
	Hypertension	0.42	0.11-1.60	.209
	Sex (male)	1.30	0.26-6.51	.74

Only statistically significant variables are reported. CCR5 Δ32 allele is an independent risk factor for dilatative arterial disease and AAA rupture.
AAA, Abdominal aortic aneurysm; *PAOD,* peripheral arterial occlusive disease; *CS,* carotid stenosis; *OR,* odds ratio; *CI,* confidence interval.

arterial lumen, whereas AAA is by definition an enlarging arterial disease. Finally, multiple logistic regression analysis was performed to identify possible independent risk factors for ruptured AAA (Table IV). Although Δ32 deletion mutation was significantly different between AAA and control groups in this series, it does not seem to be an independent risk factor for AAA ($P = .064$), but there is a trend toward significance.

When patients are considered, the presence of Δ32 allele is an independent risk factor that significantly differentiates patients with AAA from those with PAOD or carotid stenosis ($P = .012$). Furthermore, within the AAA group the Δ32 allele is an independent risk factor for rupture ($P = .045$), whereas hypertension, cigarette smoking, and gender are not involved in this life-threatening complication.

DISCUSSION

Atherosclerosis is a progressive multifactorial vascular disease characterized in part by the early and persistent presence of macrophages, T lymphocytes, and vascular dendritic cells within arterial walls.[21-24] These cellular populations are involved in the chronic inflammatory process that has a role in the pathogenesis of atherosclerosis.[25] The AAA wall is also characterized by a chronic inflammatory infiltrate.

However, there is evidence that the 2 processes affect distinct layers of the arterial wall; atherosclerosis mainly involves the inner layers, intima, and media, whereas AAA typically affects the outer layers, media, and adventitia.[26]

Inflammatory infiltration by macrophages and lymphocytes is a common underlying factor in the pathogenesis of atherosclerosis and AAA. As a consequence, both diseases may be considered a response to an injury that develops in 2 distinct directions. Atherosclerosis is linked with wall thickening and reduction of the arterial lumen, whereas AAA is linked with wall thinning and enlargement of the arterial lumen, partially attenuated by thrombus deposition.

Inflammatory cell infiltration of the arterial wall activates a cascade of enzymes involved as mediators of inflammation. Chemokines have a key role in cellular recruitment, and induction of expression and activation of several enzymes involved in extracellular matrix remodeling and metabolism, in particular, matrix metalloproteinases and their inhibitors.[25,26] CC chemokine receptors are important modulators of inflammation. Although CC chemokine receptors have been found predominantly on leukocytes, recent studies have suggested that vascular muscle cells respond to CC chemokines.[27]

Reed et al[28] described the similarity of risk factors for occlusive and aneurysmal diseases in a sample of 8000 men, and concluded that atheroma is a causal pathway to aneurysm development. The usual occlusive forms of atheroma involve intimal accumulation of material (eg, lipids, matrix proteins, cells), whereas the medial layer, which consists of

smooth muscle cells and insoluble extracellular matrix, remains largely uninjured, In contrast, aneurysm development involves proteolytic injury to the medial layer. These injuries include degradation of elastin and collagen, smooth muscle cell rarefraction, and compensatory fibrosis of the adventitia.

The change in atheroma evolution toward aneurysm development might be determined in part by genetic background.[29] Genetic susceptibility to aneurysm development from atherosclerotic disease may be associated with polymorphisms located on candidate genes. Our study proposes CCR5 polymorphism as 1 of these candidate genes. Indeed, our results show that the Δ32 deletion differentiates AAA from both PAOD and carotid stenosis.

Furthermore, almost 50% of patients with ruptured aneurysms in our series were Δ32 allele carriers; patients with AAA heterozygous for Δ32 are at 4-fold higher risk for aneurysm rupture. Although this series is not large enough to enable definitive conclusions, it is remarkable that the association between the presence of the Δ32 allele in patients with AAAs electively operated on and ruptured AAAs raises statistical significance, both for genotype distribution ($P = .011$) and allele frequency ($P = .035$), in the sense of a tendency to rupture in Δ32 carriers (Table III).

These results seem to be somewhat reinforced when the diameter of AAAs is concerned, because ruptured AAAs showed a tendency toward mean diameter smaller than electively repaired AAAs ($P = .08$). If confirmed in further, larger studies, these results would implicate CCR5 signaling as having an important role in facilitating rupture of AAAs.

Recent studies suggest that individuals homozygous for Δ32 are at reduced risk for coronary artery disease, although this allele does not have an apparent protective effect in the heterozygous form.[16]

Δ32 mutation causes truncation and loss of CCR5 receptors on lymphoid cell surface. The possibility that CCR5 ligands (regulated on activation, T-cell expressed and secreted [RANTES], macrophage inflammatory protein-1α, macrophage inflammatory protein-1β) are involved in development of atherosclerosis suggests that the absence of this receptor might reduce the risk for susceptibility to the disease. Kuziel et al[30] demonstrated that the early stages of plaque formation in apolipoprotein E knockout mice do not depend on CCR5.

Another hypothesis suggests that Th1 immune responses appear to predominate in human arterial occlusive lesions[31] and that in turn AAA predominantly express Th2-associated cytokines.[32] Correspondingly in AAA, there is a decrease in or absence of mediators associated with Th1 response, in particular the interferon-γ signaling pathway. Furthermore, it was demonstrated that Th1 CD4+ lymphocytes preferentially express CCR5 and CXCR3.[33] The results of our study suggest that in AAA there is a decrease in or absence of the CCR5-mediated pathway as well. This finding could support the possibility that immune response is involved, to some degree, in formation and development of AAA; a 40 kDa chemoattractant protein (AAAP-40) and immunoglobulin G were found within the wall of AAAs in human beings.[26,33]

Because persons with inactive CCR5 seem to be otherwise healthy, it appears that complete or partial blockage of CCR5 is not detrimental. There are no obvious abnormalities in these individuals, and the frequency of the inactive or deleted allele appears in certain populations to approach 10% to 20%. The discovery of CCR5-deprived healthy persons raises the question of what this receptor does. The answer would provide as much insight into the physiologic characteristics of chemokines as the pathophysiologic features of atherosclerosis and AAA.

REFERENCES

1. MacSweenwy STR, Powell JT, Greenhalg RM. Pathogenesis of abdominal aortic aneurysm. Br J Surg 1994;81:935-41.
2. Thompson RW. Basic science of abdominal aortic aneurysms: emerging therapeutic strategies for an unresolved clinical problem. Curr Opin Cardiol 1996;11:504-18.
3. Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalg PM, Powell JT. Inflammation and matrix metalloproteinases in enlarging abdominal aortic aneurysm. Arteioscler Thromb 1995;15:1145-51.
4. Koch AE, Haines GK, Rizzo RJ, Radosevich JA, Pope RM, Robinson PG. Human abdominal aortic aneurysms: immunophenotypic analysis suggesting an immune-mediated response. Am J Pathol 1990;137: 1199-1213.
5. Parums DV, Dunn DC, Dixon AK, Mitchinson MJ. Characterization of inflammatory cells in patients with chronic periaortites. Am J Cardiovasc Pathol 1990;3:121-9.
6. Baggiolini M. Chemokines and leukocyte traffic. Nature 1998;392: 565-8.
7. Luster AD. Chemokines: chemotactic cytokines that mediated inflammation. N Engl J Med 1998;338:436-45.
8. Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, et al. Contrasting influence of CCR2 and CCR5 variants of HIV infection and disease progression. Science 1997;277:959-65.
9. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, et al. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC). Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. Science 1998;279:389-93.
10. Barcellos LF, Schito AM, Rimmler JB, Vittinghoff E, Shihh A, Lincoln R, et al, for the Multiple Sclerosis Genetic Group: CC-chemokine receptor 5 polymorphism and age of onset in familial multiple sclerosis. Immunogenetics 1998;48:1462-5.
11. Rottman JB, Ganley KP, Williams K, Wu L, Mackay CR, Ringler DJ. Cellular localization of the chemokine receptor CCR5. Am J Pathol 1997;151:1341-51.
12. Mack M, Cihak J, Siminis D, Luckow B, Proudfoot AEI, Planchy J, et al. Expression and characterization of the chemokine receptors CCR2 and CCR5 in mice. J Immunol 2001;166:4697-4704.
13. Berger O, Gan X, Gujuluva C, Burns AR, Sulur M, Way D, et al. CXC and CC chemokine receptors on coronary and brain endothelia. Mol Med 1999;5.795-805.
14. Schecter AD, Calderon TM, Berman AB, McManus CM, Fallon JT, Rossikhina M, et al. Human vascular smooth muscle cells possess functional CCR5. J Biol Chem 2000;275:5466-71.
15. Wilcox JN, Nelken NA, Coughlin SR, Gordon D, Shall TJ. Local expression of inflammatory cytokines in human atherosclerotic plaques. J Atheroscler Thromb 1994;1:S10-3.
16. Gonzalez P, Alvarez R, Batalla A, Reguero JR, Alvarez V, Astudillo A, et al. Genetic variation at the chemokine receptors CCR5/CCR2 in myocardial infraction. Gene Immun 2001;2:191-5.

17. Szalai C, Duba J, Prohaszka Z, Kalina A, Szabo T, Nagy B, et al. Involvement of polymorphisms in the chemokines system in the susceptibility for coronary artery disease (CAD). Atherosclerosis 2001;158:233-9.
18. Biller J, Feinberg WM, Castaldo JE, Whittemore AD, Harbaugh RE, Dempsey RJ, et al. Guidelines for carotid endarterectomy: a statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Circulation 1998;97:501-9.
19. Blanchard JF, Armenian HK, Friesen PP. Risk factors for abdominal aortic aneurysm: results of a case-control study. Am J Epidemiol 2000; 151:575-83.
20. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al, and the National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.
21. Ross R. The pathogenesis of atherosclerosis: a prospective for the 1990s. Nature 1993;362:801-9.
22. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340:115-26.
23. Reape TJ, Groot PH. Chemokines and atherosclerosis. Atherosclerosis 1999;147:213-35.
24. Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001;104: 503-16.
25. Kadar A, Glasz T. Development of atherosclerosis and plaque biology. Cardiovasc Surg 2001;9:109-21.
26. Ailawadi G, Eliason JL, Upchurch GR JR. Current concepts in the pathogenesis of abdominal aortic aneurysm. J Vasc Surg 2003;38:584-8.
27. Schecter AD, Calderon TM, Berman AB, McManus CM, Fallon JT, Rossikhina M, et al. Human vascular smooth muscle cells possess functional CCR5. J Biol Chem 2000;275:5466-71.
28. Reed D, Reed C, Stemmeermann G, Hayashi T. Are aortic aneuysms caused by atherosclerosis? Circulation 1992;85:205-11.
29. Armstong PJ, Johanning JM, Calton WC JR, Delatore JR, Franklin DP, Han DC, et al. Differential gene expression in human abdominal aorta: aneurysmal versus occlusive disease. J Vasc Surg 2002;35:346-55.
30. Kuziel WA, Dawson TC, Quinones M, Garavito E, Chenaux G, Ahuja SS, et al. CCR5 deficency is not protective in the early stages of atherogenesis in apoE knockout mice. Atherosclerosis 2003;167:25-32.
31. Frostegåard J, Ulfrgren AK, Nyberg P, Hedin U, Swedenborg J, Andersson U, et al. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis 1999:145;33-43.
32. Schönbeck U, Sukhova GK, Gerdes N, Libby P. Th2 predominant immune responses prevail in human abdominal aortic aneurysm. Am J Pathol 2002;161:499-506.
33. Xia S, Ozvath K, Hirose H, Tilson MD. Partial amino acid sequence of a novel 40-kDa human aortic protein, with birtonectin-like, fibrinogen-like, and calcium binding domains: aortic aneurysm-associated protein-40 (AAAP-40). Biochem Biophys Res Comm 1996;219:36-9.

Submitted Jan 9, 2004; accepted Aug 8, 2004.